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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Microbiological Research Authority CAMR Porton Down Salisbury Wiltshire SP4 0JG	
Patents ADP number *(if you know it)*	6566160001	
If the applicant is a corporate body, give the country/state of its incorporation	Great Britain	
4. Title of the invention	Modulation Of C-Fibre Activity	
5. Name of your agent *(if you have one)*		
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MODULATION OF C-FIBRE ACTIVITY

The present invention relates to treatment of pain and to compounds that modulate C-fibre activity, in particular the use of these compounds for the treatment of diseases of which C-fibre activity is a component, such as pain, inflammation, psoriasis and other C-fibre related conditions, to methods and compositions therefor and manufacture of those compositions.

A number of disease conditions are known to be related to or associated with activity in C-fibre neurones, in particular pain, inflammation and psoriasis.

C-fibres are small diameter, unmyelinated neurones, which have their sensory termini and cell bodies in the peripheral nervous system (PNS), but which synapse in the dorsal horn of the spinal cord within the central nervous system (CNS). The accepted role of these afferent fibres is to signal strong, injury-threatening (noxious) stimuli or the presence of chemical irritants, including many inflammatory mediators to the CNS. The neurones are thus termed nociceptors. However, the importance of C-fibre neurons in a variety of other clinical conditions is only now emerging.

Uniquely amongst afferent neurones, many C-fibre nociceptors also have efferent, antidromic actions within the peripheral tissue that they innervate. When stimulated, the C-fibres release vasoactive mediators, which have potent actions on local blood vessels and on cells of the immune system. This invariably results in the phenomenon termed neurogenic inflammation. C-fibre efferent neurones store and co-release upon stimulation the neuropeptides substance P (SP) and calcitonin gene related peptide (CGRP). Both of these peptides are potently vasoactive and the blood vessel dilation by CGRP combined with an increase in SP induced vascular permeability results in plasma extravasation and peripheral oedema. This in turn recruits infiltration by eosinophils and other inflammatory cells and subsequently the full panoply of an inflammatory cascade.

Most citations report neurogenic inflammation in the skin. However, C-fibres are distributed widely throughout the body. In bronchial airway tissues, substances released from nociceptive terminals may cause smooth muscle contraction and also trigger the secretion of mucus. Detailed information is also available for C-fibre mediated effects in the bladder and the gastrointestinal tract. Neurogenic inflammation has now been clearly associated with a variety of disorders including asthma, ulcer formation and headache. It may also be a component of generalised inflammatory responses such as arthritis.

Psoriasis is a hyperproliferative epidermal disease whose aetiopathogenesis remains largely undefined. However, one of the most favoured hypothesises is that the condition correlates with an altered release of SP and CGRP neuropeptides by sensory efferent neurones.

Studies of neurogenic inflammation in psoriatic skin have demonstrated that morphological contact between mast cells and sensory C-fibres increases in developing (1-3 weeks) psoriatic lesions when compared to non-lesional controls. This increase reaches statistical significance in mature lesions. It is considered that the associated increase in neurogenic stimulation promotes mast cell degranulation and the release of inflammatory mediators. This in turn allows entry into the lesional dermis of various mediators that interact with keratinocytes to promote the hyperproliferation characteristic of psoriatic plaques.

A variety of psoriasis treatments are available ranging from moisturising creams, phototherapy and topical vitamin applications to corticosteroids and immune suppressants. However, none of these are universally effective nor do they appear to treat the underlying causes of the disease. Furthermore, the most effective treatments, especially for conditions requiring intensive therapy, have unpleasant and often toxic side effects.

Pain can be classified in a number of ways depending upon the cause, the mechanism of action and the duration of suffering. Pain can also be categorised by the level of discomfort, *e.g.* mild, moderate or severe. Most pain is caused by direct mechanical, thermal or chemical damage to specific sensory receptors (nociceptors) which are located in the skin, muscle, some viscera and a number of other tissues. The perception of "feeling pain" follows the transduction and transmission of a noxious stimulus from the site of this insult or damage to the higher centres of the Central Nervous System (CNS).

Transduction of the majority of pain signals arises from the activation of small diameter, thinly myelinated or non-myelinated neurones known as A_{δ}- and C-fibres respectively. These fibres have their cell bodies in the dorsal root ganglia, juxtaposed along the length of the spinal column. They synapse primarily in dorsal horn laminae I and II of the spinal cord. The most common nociceptors are innervated by C-fibres.

The transmission of a pain stimulus (nociception) is a complex process. There are many chemical transmitters involved at the site of insult and throughout the Peripheral Nervous System (PNS) and CNS. For each of these chemical transmitters there is also an associated receptor, or group of receptors, which transduce and transmit the pain impulse.

Receptors can be designated as either excitatory or inhibitory. Activation of excitatory receptors causes neuronal firing, resulting in either an increase in neurotransmitter release or an increase in the excitability of neurones. In contrast, activation of inhibitory receptors causes a decrease in neuronal firing, a reduction in transmitter release and may cause a reduction in neurone excitability.

The interaction of pain neurotransmitters with their receptors provides a large number of potential intervention points for novel therapies for the treatment

of pain. Each element, receptor, chemical mediator, neurotransmitter, ion channel and synapse, in this complex pathway is a potential target for blockade.

The current analgesic standards are the opiates, for example morphine. While these compounds are effective in a range of pain conditions, they do not provide a panacea. Their effects are short lived, thus requiring frequent application. Furthermore, there are associated unpleasant side effects of respiratory depression, nausea, dependency, addiction and induction of tolerance.

Activity of C-fibres is also implicated in the modulation of mucus release in disease conditions such as those involving mucus hypersecretion.

It is an object of the present invention to provide new methods and compositions for treatment of pain and for treatment of C-fibre related disease conditions such as inflammation, psoriasis, mucus hypersecretion and pain.

Accordingly, a first aspect of the invention provides the use of a lectin in manufacture of a medicament for modulation of C-fibre neurone activity. The first aspect of the invention also provides a method of modulating C-fibre activity by administering an effective amount of lectin.

In use of an embodiment of the invention, a lectin administered in a pharmaceutical composition binds to a C-fibre neurone and inhibits transmission of nerve cell impulses via that neurone, treating and thereby reducing a disease condition caused by or involving excessive activity of that C-fibre neurone.

Oligosaccharide carbohydrate receptors regulate key stages in many physiological processes of pharmaceutical importance such as inflammation, oncology, immunology, neural, infectious and other types of diseases

(Edmonds, B, J. and Koenig, E. [1990] *Cell Motil. Cytoskeleton*, **17**, 106-17; Edmonds, B, J. and Koenig, E. [1991] *J. Neurocytol.* **20**, 232-47; Gupta, D., Arango, R. and others [1994] *Biochemistry*, **33**, 2503-08). Lectins are a class of proteins, often glycoproteins, that bind to carbohydrate structures and are known to agglutinate cells and/or precipitate complex carbohydrates. Two publications describing how lectins bind to neuronal cells are Silverman, J. D. and Kruger, L. (1990), selective neuronal glycoconjugate expression in sensory and autonomic ganglia: relation of lectin reactivity to peptide and enzyme markers, *J. Neurophysiol.* **19**, 789-801; and Streit, W.J., Schulte, B.A., Balentine, D. and Spicer, S.S. (1985), histochemical localization of galactose-containing glycoconjugates in sensory neurons and their processes in the Central and Peripheral Nervous System of the rat, *J. Histochem. Cytochem.* **33**, 1042-52. Lectins can be isolated from a wide range of natural sources including seeds, plant roots and bark, fungi, bacteria, seaweed and across the whole range of life forms from viruses to mammals. Some antibodies are lectins, though the most commonly exploited sources are the abundant lectins found in the seeds of plants. The precise physiological role of lectins in nature is still unknown, but they have proved to be very valuable in a wide variety of applications *in vitro* including blood grouping and histochemical staining for cell surface markers. It is known in the literature that lectins have demonstrable selectivity for cells of different origin.

Due to the ubiquitous nature of oligosaccharide structures, lectins bind to a large number of cells. However, because of the known complexity and specificity of the transduction and transmission pathways of C-fibre neurones, it is particularly surprising and unexpected that lectins can bind to neurones so as to modulate C-fibre activity. For the purposes of this invention a "lectin" or a "lectin domain" is the whole of or that part of a peptide, protein or glycoprotein that has the ability to bind specifically to mono- or oligosaccharide structures. The invention is of application without limit to particular sub-groups of lectins, though preferred lectins are those that bind galactosyl residues, for example terminal β-D-galactosyl or terminal α-D-

galactosyl residues or N-acetylagalactosamine residues, or glucosyl residues.

In use of an embodiment of the invention, a lectin binds to a surface structure or structures (the Binding Site [BS]) which is characteristic of, and has a degree of specificity for relevant sensory effector cells and/or neurones in the peripheral or central nervous system responsible for the afferent or projection transmission of pain or the regulation of said transmission. Next, it modulates the function of such cells so that either the transduction or transmission of the pain signal is reduced or abolished. Such modulation may be due to suppression of the activity of afferent and or projection neurones or due to promotion of the activity of inhibitory neurones.

One aspect of the invention thus advantageously provides a further class of analgesic compositions and uses of these compositions for treatment of pain. The invention provides use of a lectin in manufacture of a medicament for treatment of pain and a method of treatment of pain comprising administration of an effective amount of a lectin. In specific embodiments of the invention, lectins have been tested using the mouse hot plate model - an industry standard model acknowledged as predictive of human response - and found to be analgesic. The mouse hot-plate model is acknowledged as a model for human pain in Heilman, R. D. *et al.* (1976), An evaluation of the hot plate technique to study narcotic analgesics. *Res. Comm. Chem. Pathol. Pharmacol.* **13 (4)**, 635-647.

In a specific embodiment of the invention a galactoseries-specific lectin is extracted from the coral tree *Erythrina cristagalli* and administered intrathecally, exhibiting strong analgesic activity. In a further specific embodiment of the invention a galactoseries-specific lectin is extracted from the coral tree *Erythrina corallodendron* and administered intrathecally, also exhibiting strong pain-reducing activity. These illustrative lectins are heterodimeric and homodimeric glycoproteins respectively of approximate molecular weight of 60 kDa (Arango, R. and others [1992] *Eur. J. Biochem.*

205, 575-81). In a still further specific embodiment of the invention, a glucosyl - specific lectin is analgesic in the mouse hot plate model.

A list of commercially available lectins appears in the 1998 Sigma catalogue. A further example of a lectin, specifically a bacterial lectin is PA-I from *Pseudomonas aeruginosa*. This is a D-galactose binding lectin with a molecular weight of 13 kDa (Garber, N., Guempel, U. and others (1992). *Biochim. Biophys. Acta.* **1116,** 331-3).

In use of a further embodiment of the invention, a lectin binds to a C-fibre neurone that innervates a psoriatic lesion and inhibits release of inflammatory modulators, including substance P. A further aspect of the innovation thus advantageously provides a class of compositions for treatment of psoriasis.

In use of a still further embodiment of the invention, a lectin binds to a C-fibre neurone which modulates mucus release or control of mucus release. A still further aspect of the invention thus advantageously provides a class of composition and uses thereof for treatment of mucus hypersecretion.

Site and mode of delivery of lectin is typically a factor in obtaining the desired therapeutic effect. Thus, a topically and/or specifically delivered composition containing a lectin can comprise a lectin that binds to C-fibres but is not specific for C-fibres. By way of example, an embodiment of the invention provides for treatment of a localised disease condition such as psoriasis, using a lectin formulated in a cream which can be topically applied or a lectin formulated for sub-dermal injection. In this way, delivery of a lectin according to the invention is targeted and not systemic. In the case particularly of formulations for injection, it is optional to include a further pharmaceutically active substance to assist retention at or reduce removal of the lectin from the site of administration, and one example is the use of a vasoconstrictor such as adrenaline. Such a formulation confers the advantage of increasing the residence time of lectin following administration and thus increasing and/or

enhancing its effect.

Similar targeting delivery is appropriate for diseases involving C-fibre activity and for which the active lectin can be delivered via aerosol or other spray. An aerosol formulation of a lectin enables delivery to the lungs and/or other nasal and/or bronchial or airway passages, facilitating use of lectin for treatment of diseases such as mucus hypersecretion and rhinitis (hay fever).

Generally, the invention is of application to treatment of diseases caused by or exacerbated by antidromic activity in C-fibres, by inhibition of the antidromic activity, which is typically at least partially self-perpetuating or of an origin that is not clearly defined or presently understood. More specifically, the compounds and formulations of the invention can be used for treatment of inflammation including neurogenic inflammation, erythema, irritable bowel syndrome, headache, asthma, arthritis and as already mentioned pain including migraine.

Lectins and lectin domains may be arranged, covalently or non-covalently, so as to form oligomers and/or polymers, their functional units optionally separated using linkages which may include one or more spacer arms. The chemical conjugation and genetic fusion of such domains can be achieved by conventional methods. Lectins and lectin domains may be coupled to a distinct, additional, lectin or non-lectin companion protein forming a multi-component agent such that the efficacy of the agent is enhanced when compared to the native lectin component alone. Such an agent is optionally the expression product of a recombinant gene which provides for a minimal functional peptide domain such that the agent may be monomeric in form and may optionally be glycosylated. The agent may be the expression product of a recombinant gene delivered independently to the preferred site of action of the agent. Gene delivery technologies are widely reported in the literature (reviewed in "*Advanced Drug Delivery Reviews*" Vol. 27, [1997, Elsevier Science Ireland Ltd].

Methods for extracting and purifying lectins are known in the literature, for example, Iglesias, J. L., Lis, H. and Sharon, N. (1982) *Eur. J. Biochem.* **123**, 247-252.

Gene expression technologies for the provision of functional, recombinant lectins, both glycosylated and non-glycosylated, are known in the literature (Arango, R. and others [1992] *Eur. J. Biochem.* **205**, 575-81). By way of example, a cDNA encoding a lectin can be cloned into any of the pET series of plasmid vectors. Expression of the gene is thus under control of the T7 promoter and can be well regulated in the *E. coli* host. The expression product is recovered and purified from inclusion bodies using established techniques. These methods have been shown to produce non-glycosylated lectin proteins that otherwise retain all of the known functions of the native lectins.

Recombinant lectin protein fusions may be made by a variety of approaches. For example, the fusion of a gene coding for a lectin to a gene coding for a second lectin or non-lectin protein. These gene fusions may be separated by a further nucleic acid sequence coding for a flexible linker region (*e.g.* a polyglycine tract) which facilitates interaction of the fused proteins either with themselves (oligomerisation) or with other target molecules (receptors, other lectins etc.).

The lectin may further be expressed in single copy or multicopy arrangements on the surface of bacteriophage such that the monomers may associate to form a functional oligomeric molecule. In some instances, functionality may request the association of two or more separate bacteriphage displaying or require expressing lectin monomers. This approach may also be used for the expression of lectin fusion proteins.

The present invention further provides a composition comprising a lectin as described coupled to another peptide or protein. The peptide or protein may

be a carrier or may impart a second function to the composition in addition to the analgesic properties of the lectin. The present invention further provides a composition comprising a first lectin conjugated to a second lectin, the lectins being optionally different or the same. They can be conjugated directly or via an intermedite linker. The lectins of the invention may also be modified to make derivatives, for example modified to remove a carbohydrate group whilst maintaining its ability to bind target cells.

The protein is an endopeptidase in one embodiment of the invention, suitably of clostridial origin. Specifically, the lectin of the invention can be coupled to an endopeptidase from a clostridial species, optionally from botulinum neurotoxin type A, or B. Such compositions are conveniently made by coupling a galactose-binding lectin to an enzymatically active fragment of a clostridial neuro toxin, for example an LH_N fragment of a botulinum neurotoxin, and in an illustrative example set out below a galactose-binding lectin from *Erythrina cristagalli* is coupled to an LH_N fragment of botulinum neurotoxin type A. The present invention thus still further provides a method of preparing a composition of the invention by covalently attaching a lectin to another peptide or protein.

The lectin of the invention either in isolated form or incorporated into compounds or compositions may exert its activity by preventing the release of a neurotransmitter or neuromodulator from a primary sensory afferent, by inhibiting the release of a neurotransmitter or neuromodulator from a primary nociceptive afferent, by inhibiting the release of a neurotransmitter or neuromodulator from a projection neurone, by promoting the activity of an inhibitory neurone or by a combination thereof.

Another aspect of the invention lies in a method of modulating C-fibre activity comprising administering an effective amount of a lectin to a patient. The lectin can be used to inhibit C-fibre activity, or to stimulate C-fibre activity.

Further aspects of the invention lie in a composition for modulation of C-fibre activity comprising a nucleic acid encoding a lectin, a method of modulating C-fibre activity comprising administering an effective amount of such a composition, and use of such a composition in manufacture of a medicament for modulation of C-fibre activity.

In a yet further aspect, the invention provides a pharmaceutical composition for modulation of C-fibre neurone activity, comprising

a lectin or a composition according to any of the above aspects of the invention; and

at least one of a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and or salt.

The compound may thus be formulated for oral, parenteral, continuous infusion, inhalation or topical application and encompasses delivery of the lectin, its nucleic acid coding sequence or its agonists. Intraspinal injection is another route of administration, though the present invention encompasses also any administration that delivers the compound to an appropriate or optimal site of intervention. Administration might take advantage of a variety of delivery technologies including microparticle encapsulation, viral delivery systems or high pressure aerosol impingement, including delivering a lectin-coding nucleic acid to a cell population in which it would be expressed and the gene product would be capable of effecting analgesia. Additionally, the target cells for lectin activity need not be the cells in which the gene is expressed.

Agents of the invention for treatment of pain may be administered to a patient by intrathecal or epidural injection in the spinal column at the level of the spinal segment involved in the innervation of an affected organ. This is, for example, applicable in the treatment of deep tissue pain, such as chronic malignant pain. The dosage ranges for administration of the compounds of the present invention are those to produce the desired therapeutic effect. It will

be appreciated that the dosage range required depends on the precise nature of the lectin or composition, the route of administration, the nature of the formulation, the age of the patient, the nature, extent or severity of the patient's condition, contraindications, if any, and the judgement of the attending physician. Wide variations in the required dosage, however, are to be expected depending on the precise nature of the agent. Variations in these dosage levels can be adjusted using standard empirical routines for optimisation.

Fluid dosage forms are typically prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and or local anaesthetic agents may be dissolved in the vehicle.

Dry powders, which are dissolved or suspended in a suitable vehicle prior to use, may be prepared by filling pre-sterilised drug substances and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Compositions suitable for administration via spinal injection may be made up in a conventional manner and employed in conjunction with conventional administration devices.

Compositions of the previously filed International patent application PCT/GB98/03001 that contain lectins may be used in the present invention but are excluded from the scope of compositions claimed herein.

There now follows description of specific embodiments of the invention, illustrated by drawings in which:

Fig. 1 shows a denaturing polyacrylamide gel analysis of ECL lectin, LH_N/A companion protein and the subsequent chemical conjugates;

Fig. 2 shows the analgesic effect of lectin in mice in a hot-plate model;

Fig. 3 shows the analgesic effect of lectin-conjugates in mice in a hot-plate model;

Fig. 4 shows WGA lectin is analgesic in the mouse hot plate model; and

Fig. 5 shows lectin IB_4 is analgesic in the mouse hot plate model.

Example 1.

Preparation of *Erythrina cristagalli* lectin.

Lectins from *E. cristagalli* may be prepared as described by Iglesias, J. L., Lis, H. and Sharon, N. (1982) *Eur. J. Biochem.* **123**, 247-252. Alternatively the purified lectin may be purchased from a range of suppliers such as Sigma.

Example 2.

Method for the preparation of oligomers of *Erythrina cristagalli* lectin.

Materials

E. cristagalli lectin (ECL) was obtained from Sigma Ltd. SPDP was from Pierce Chemical Co. PD-10 desalting columns were from Pharmacia. Dimethylsulphoxide (DMSO) was kept anhydrous by storage over a molecular sieve. Denaturing sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using gels and reagents from Novex. Immobilised lactose-agarose was obtained from Sigma Ltd. Additional reagents were obtained from Sigma Ltd.

Methods

The lyophilised lectin was rehydrated in phosphate buffered saline (PBS; 10mM Na_2HPO_4, 140mM NaCl, 2.7mM KCl, 1.8mM KH_2PO_4, pH7.3) to a final concentration of 10 mg/ml. Aliquots of this solution were stored at -20°C until used.

For conjugation the lectin was reacted with an equal concentration of SPDP by the addition of a 10 mM stock solution of SPDP in DMSO with mixing. After one hour at room temperature the reaction was terminated by desalting into PBS over a PD-10 column.

From one aliquot of the SPDP derivatised lectin material the thiopyridone leaving group was removed by reduction with dithiothreitol (DTT, 5 mM, 30 min). The product of this reaction was analysed spectrophotometrically at 280 nm and 343 nm to determine the degree of derivatisation achieved. The degree of derivatisation achieved was 0.8 ± 0.06 mol/mol. The thiopyridone and DTT were removed by once again desalting into PBS over a PD-10 column.

A second aliquot of SPDP derivatised lectin material protein was desalted into PBSE (PBS containing 1 mM EDTA).

The bulk of the derivatised ECL lectin and the DTT treated derivatised ECL were mixed in proportions such that the DTT treated derivatised ECL was in

greater than three-fold molar excess. The oligomerisation reaction was allowed to proceed for >16 h at 4°C.

The product mixture was centrifuged to clear any precipitate that had developed. The supernatant was concentrated by centrifugation through concentrators (with 10000-50000 molecular weight exclusion limit), dialysed against PBS, and stored at 4°C until use.

The oligomerisation products were analysed by SDS-PAGE on 4-20% polyacrylamide gradient gels, followed by staining with Coomassie Blue.

Example 3.

Method for the preparation of a conjugate between *Erythrina cristagalli* lectin and a companion protein.

Materials

E. cristagalli lectin (ECL) was obtained from Sigma Ltd. The companion protein in this example is a clostridial enzyme designated LH_N/A and was prepared essentially by the method of Shone C.C., Hambleton, P., and Melling, J. 1987, *Eur. J. Biochem.* **167**, 175-180. SPDP was from Pierce Chemical Co. PD-10 desalting columns were from Pharmacia. Dimethylsulphoxide (DMSO) was kept anhydrous by storage over a molecular sieve. Denaturing sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using gels and reagents from Novex. Immobilised lactose-agarose was obtained from Sigma Ltd. Additional reagents were obtained from Sigma Ltd.

Methods

The lyophilised lectin was rehydrated in phosphate buffered saline (PBS; 10mM Na_2HPO_4, 140mM NaCl, 2.7mM KCl, 1.8mM KH_2PO_4, pH7.3) to a final concentration of 10 mg/ml. Aliquots of this solution were stored at -20°C until

used.

The lectin was reacted with an equal concentration of SPDP by the addition of a 10 mM stock solution of SPDP in DMSO with mixing. After one hour at room temperature the reaction was terminated by desalting into PBS over a PD-10 column.

The thiopyridone leaving group was removed from the product by reduction with dithiothreitol (DTT, 5 mM, 30 min). The product of this reaction was analysed spectrophotometrically at 280 nm and 343 nm to determine the degree of derivatisation achieved. The degree of derivatisation achieved was 0.8 ± 0.06 mol/mol. The thiopyridone and DTT were removed by once again desalting into PBS over a PD-10 column.

The companion protein was desalted into PBSE (PBS containing 1 mM EDTA). The resulting solution (0.5-1.0 mg/ml) was reacted with a four- or five-fold molar excess of SPDP by addition of a 10 mM stock solution of SPDP in DMSO. After 3 h at room temperature the reaction was terminated by desalting over a PD-10 column into PBS.

A portion of the derivatised companion protein was removed from the solution and reduced with DTT (5 mM, 30 min). This sample was analysed spectrophotometrically at 280 nm and 343 nm to determine the degree of derivatisation. The degree of derivatisation achieved was 2.26 ± 0.10 mol/mol. The bulk of the derivatised companion protein and the derivatised ECL were mixed in proportions such that the ECL was in greater than three-fold molar excess. The conjugation reaction was allowed to proceed for >16 h at 4°C.

The product mixture was centrifuged to clear any precipitate that had developed. The supernatant was concentrated by centrifugation through concentrators (with 10000-50000 molecular weight exclusion limit) prior to

a two step purification strategy. As the first step, the concentrated material was applied to a Superose 12 column on an FPLC chromatography system (Pharmacia). The column was eluted with PBS and the elution profile followed at 280 nm.

Fractions were analysed by SDS-PAGE on 4-20% polyacrylamide gradient gels, followed by staining with Coomassie Blue. The major band of conjugate has an apparent molecular mass of between 130-160 kDa; this is separated from the bulk of the remaining unconjugated companion protein and more completely from the unconjugated ECL. Fractions containing conjugate were pooled prior to the second chromatography step; immobilised lactose-agarose. Selected post-Superose-12 fractions were applied to PBS-washed lactose-agarose and incubated for 2 hours at 4°C to facilitate binding. Lectin-containing protein conjugates remained bound to the agarose during subsequent washing with PBS to remove contaminants. Lectin conjugate was eluted from the column by the addition of 0.3M lactose (in PBS) and the elution profile followed at 280 nm. The fractions containing conjugate were pooled, dialysed against PBS, and stored at 4°C until use. Figure 1 shows a typical SDS-PAGE profile of ECL lectin, companion LH_N/A and the conjugation products at different stages during purification.

Example 4.

The production of a conjugate between a lectin from *Erythrina corallodendron* and a companion protein.

The procedure for production of a conjugate between a lectin from *Erythrina corallodendron* and LH_N/A is essentially as described in Example 3 but with the following differences:

Materials
Lectin from *E. corallodendron* (ECorL) was obtained from Sigma Ltd.

Example 5.

In vivo analgesic effects of *Erythrina cristagalli* lectin

Animals

Adult outbred mice (MF1) of either sex, weight range 20 to 30g have been used for these studies. All animals are acclimatised for a minimum of 4 days by maintaining them in the room in which the tests are to be carried out. Pre-injection data is acquired from all animals on the day before test substances are introduced and this serves as baseline data for individual animals. In addition, this pre-test data allows screening of animals exhibiting extreme responses to the various stimuli. Experiments are done on groups of 10 animals with replicate readings taken at each test point.

Intrathecal administration.

Mice are anaesthetised by induction with 4% fluothane carried in a 50:50 mixture of oxygen and nitrous oxide with a flow rate of 400-500cc per minute. Once anaesthesia is induced the percentage is reduced to 1.5 to 2% for maintenance.

The pelvis of the mouse is located, the fur is clipped around the appropriate area and a small incision (about 5mm) is made in the skin above the spinal column.

Using a 30 gauge disposable needle attached to a 50μl Hamilton syringe with luer fitting the lectin material is injected into the intrathecal space. The site of injection is normally chosen to be between lumbar vertebrae 5 and 6. Holding the syringe at an angle of about 20° above the vertebral column the needle is inserted into the tissue to one side of the vertebrae so that it slips into the groove between the spinous and transverse processes. The needle is then moved carefully forward to the intervertebral space whilst decreasing the angle of the syringe to about 10°. Once inserted into the appropriate

spot a characteristic flick of the tail is often, but not invariably, observed. 5μl of test material is then injected into the intrathecal space and the needle withdrawn. The skin incision is then closed with a single wound clip and the animal placed in a box to allow recovery. Mice have been observed to recover rapidly from this procedure and become fully mobile within two minutes.

E. cristagalli lectin agents were applied intrathecally at 30μg in a 5μl volume of physiological saline vehicle. The data in Figure 2 shows a rapid onset of lectin induced analgesia which approaches a maximum within 1 hour of application and remains constant for at least 5 hours. Lectin induced analgesia is similar to that of a 10μg/mouse supramaximal (20 times the mouse EC50) of morphine in this test but is of much longer duration; morphine achieves a maximal effect at 1 hour and then declines over a period of 5 hours.

Example 6.

***In vivo* analgesic effects of *Erythrina cristagalli* lectin conjugates.**

Animals

Adult outbred mice (MF1) of either sex, weight range 20 to 30g have been used for these studies. All animals are acclimatised for a minimum of 4 days by maintaining them in the room in which the tests are to be carried out. Pre-injection data is acquired from all animals on the day before test substances are introduced and this serves as baseline data for individual animals. In addition, this pre-test data allows screening of animals exhibiting extreme responses to the various stimuli. Experiments are done on groups of 10 animals with replicate readings taken at each test point.

Intrathecal administration.

Mice are anaesthetised by induction with 4% fluothane carried in a 50:50 mixture of oxygen and nitrous oxide with a flow rate of 400-500cc per

minute. Once anaesthesia is induced the percentage is reduced to 1.5 to 2% for maintenance.

The pelvis of the mouse is located, the fur is clipped around the appropriate area and a small incision (about 5mm) is made in the skin above the spinal column.

Using a 30 gauge disposable needle attached to a 50μl Hamilton syringe with luer fitting the lectin material is injected into the intrathecal space. The site of injection is normally chosen to be between lumbar vertebrae 5 and 6. Holding the syringe at an angle of about 20° above the vertebral column the needle is inserted into the tissue to one side of the vertebrae so that it slips into the groove between the spinous and transverse processes. The needle is then moved carefully forward to the intervertebral space whilst decreasing the angle of the syringe to about 10°. Once inserted into the appropriate spot a characteristic flick of the tail is often, but not invariably, observed. 5μl of test material is then injected into the intrathecal space and the needle withdrawn. The skin incision is then closed with a single wound clip and the animal placed in a box to allow recovery. Mice have been observed to recover rapidly from this procedure and become fully mobile within two minutes.

E. cristagalli lectin-LH_N/A conjugates were applied intrathecally at 30μg in a 5μl volume of physiological saline vehicle. The data in Figure 3 shows a rapid onset of lectin-conjugate induced analgesia which approaches a maximum within 1 hour of application and remains constant for at least 5 hours. Lectin-conjugate analgesia is similar in intensity to that of a 10μg/mouse supramaximal (20 times the mouse EC50) of morphine in this test but is of much longer duration; morphine achieves a maximal effect at 1 hour and then declines over a period of 5 hours.

Example 7.

In vivo analgesic effects of a glucosyl-reactive lectin from *Triticum vulgaris*.

Materials

Triticum vulgaris wheat germ agglutinin lectin (WGA) was obtained from Sigma Ltd.

Methods

Intrathecal administration of *Triticum vulgaris* lectin WGA was as described for Examples 5 and 6.

The data in Figure 4 shows that by 24-30 hours post application the hot-plate paw withdrawal time in WGA treated mice is much greater than that of PBS treated control groups. By 48 hours post application the analgesic effect from the single dose has dissipated.

Example 8.

In vivo analgesic effects of a galactosyl-reactive lectin IB_4 from *Bandeiraea simplicifolia*.

Materials

Bandeiraea simplicifolia lectin IB_4 was obtained from Sigma Ltd.

Methods

Intrathecal administration of *Bandeiraea simplicifolia* lectin IB_4 was as described for Examples 5 and 6.

The data in Figure 5 shows the analgesic effect of a single intrathecal dose of lectin IB_4 reagent in a mouse hot plate model over a 10 hour period. A significant increase in withdrawal latency is observed at 1 hour post

application with an apparent maximal activity at 4 hours. Analgesia was still clearly discernible over control-group animals at 10 hours post application..

The invention thus provides lectins and lectin derivatives useful *inter alia* for the treatment of pain and components for synthesis of molecules as described in WO 94/21300 and WO 96/33273A, and in co-pending application GB 9721189.0.

CLAIMS

1. Use of a lectin in manufacture of a medicament for modulation of C-fibre neurone activity.

2. Use according to Claim 1 for inhibiting C-fibre activity.

3. Use according to Claim 1 or 2 of a lectin specific for galactosyl residues.

4. Use according to Claim 1 or 2 of a lectin specific for glucosyl residues.

5. Use according to Claim 1 for stimulating C-fibre activity.

6. Use according to any previous Claim wherein the medicament is for treatment of pain.

7. Use according to any of Claims 1 to 5 wherein the medicament is for treatment of inflammation.

8. Use according to any of Claims 1 to 5 wherein the medicament is for treatment of psoriasis.

9. Use according to any of Claims 1 to 5 wherein the medicament is for treatment of mucus hypersecretion.

10. A composition comprising a lectin coupled to another peptide or protein.

11. A composition according to Claim 10 comprising a first lectin conjugated to a second lectin.

12. A composition according to Claim 11 wherein the first and second lectins are different.

13. A composition according to claim 10, comprising a lectin derivative, wherein the derivative has been modified to remove a carbohydrate group whilst maintaining its ability to bind C-fibres.

14. A composition according to claim 10, comprising a lectin conjugated to an endopeptidase.

15. A composition for modulation of C-fibre activity, comprising a lectin and being free of Clostridial toxin or a fragment thereof.

16. A composition according to Claim 15 for treatment of a disease or condition selected from pain, psoriasis, inflammation and mucus hypersecretion.

17. A method of modulating C-fibre activity comprising administering an effective amount of a lectin to a patient.

18. A method according to Claim 17 for inhibiting C-fibre activity.

19. A method according to Claim 17 for stimulating C-fibre activity.

20. A method according to any of Claims 17 to 19 for treating a disease or condition selected from pain, psoriasis, inflammation and mucus hypersecretion.

21. A composition for modulation of C-fibre activity comprising a nucleic acid encoding a lectin.

22. A composition according to Claim 21 for inhibition of C-fibre activity.

23. A composition according to Claim 21 for stimulation of C-fibre activity.

24. A composition according to any of Claims 21 to 23 further comprising a pharmaceutically acceptable carrier.

25. A method of modulating C-fibre activity comprising administering an effective amount of a composition according to any of Claims 21 to 23.

26. Use of a composition according to any of Claims 21 to 23 in manufacture of a medicament for modulation of C-fibre activity.

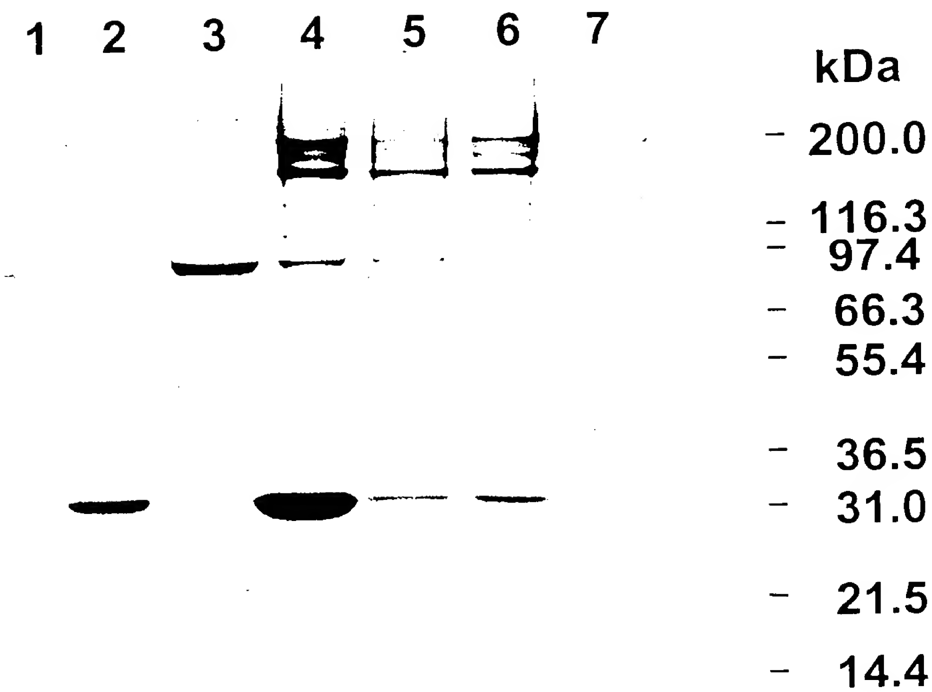

Figure 1.
Denaturing polyacrylamide gel analysis of ECL lectin, LH_N/A companion protein and the subsequent chemical conjugates.

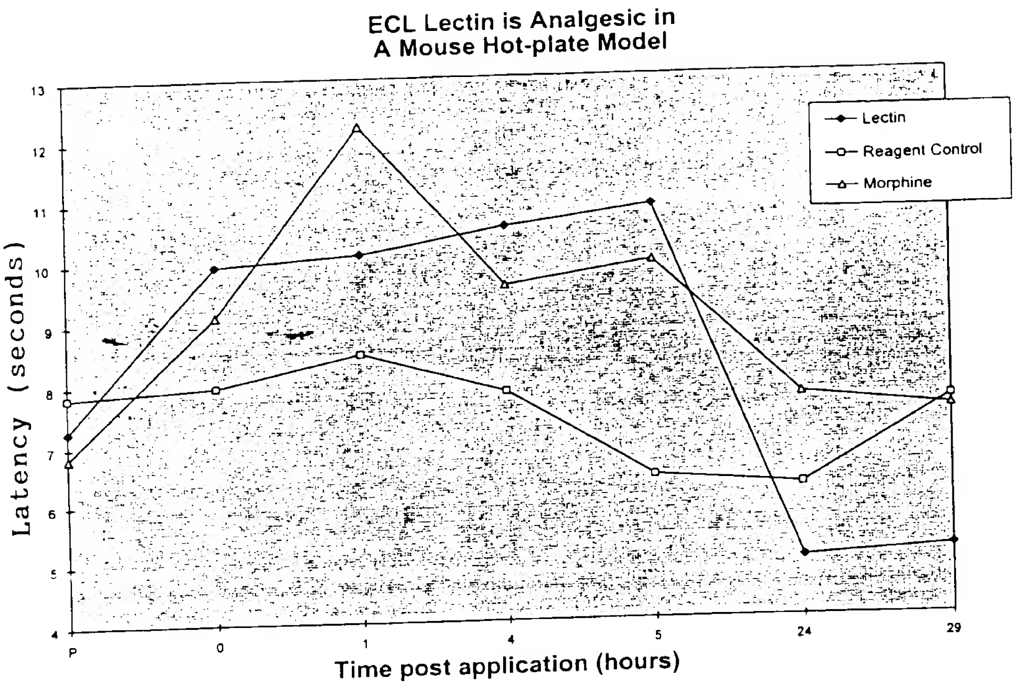

Figure 2
Erythrina cristagalli lectin is analgesic in a mouse hot-plate model
Lectin was applied intrathecally (30µg in a 5µl vehicle volume). Onset of lectin-induced analgesia reaches a plateau at 1 hour post application and remains constant for at least 5 hours. Morphine achieves a maximal effect at 1 hour and then returns to control levels over a period of 5 hours. (Data is the mean of replicate readings from groups of 10 mice)

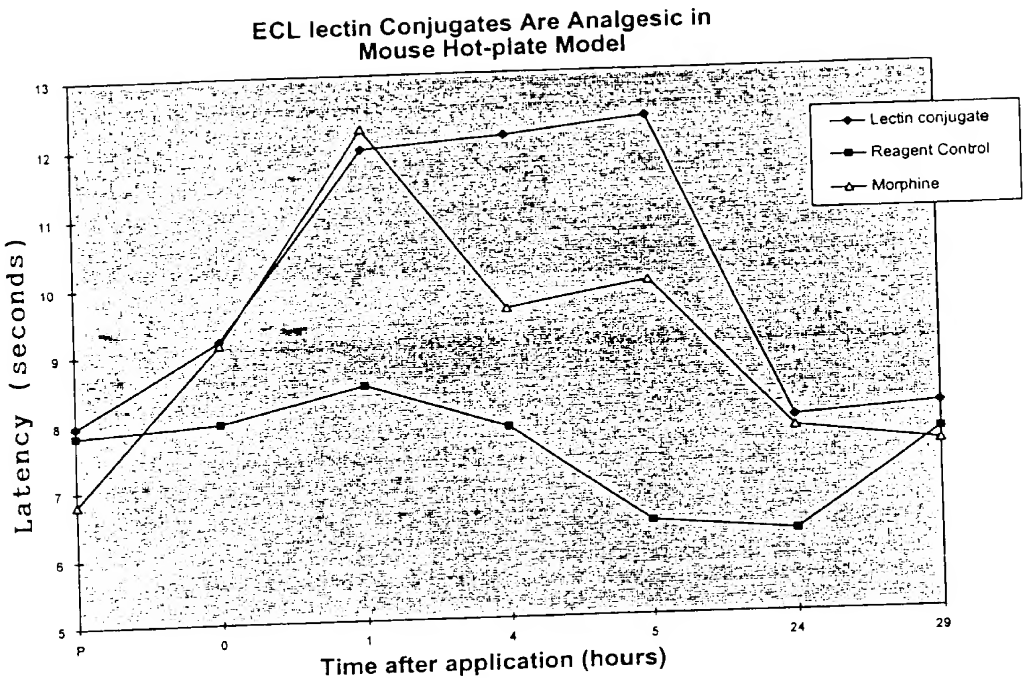

Figure 3
Lectin-conjugate induced analgesia is comparable to a supramaximal dose of morphine in a hot-plate model of analgesia
Lectin-conjugate was applied intrathecally (30µg in a 5µl vehicle volume). Onset of lectin-conjugate induced analgesia reaches a plateau at 1 hour post application and remains constant for at least 5 hours. Lectin-conjugate analgesia is similar to a supramaximal dose (20X EC50) of morphine in this test, but is of much longer duration; morphine achieves a maximal effect at 1 hour and then returns to control levels over a period of 5 hours. (Data is the mean of replicate readings from groups of 10 mice)

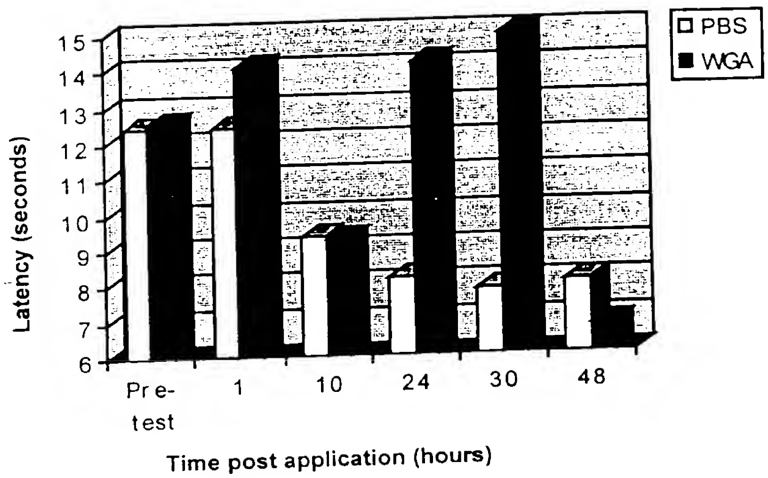

Figure 4.
Wheat germ agglutinin (WGA) lectin is analgesic in a mouse hot plate model.

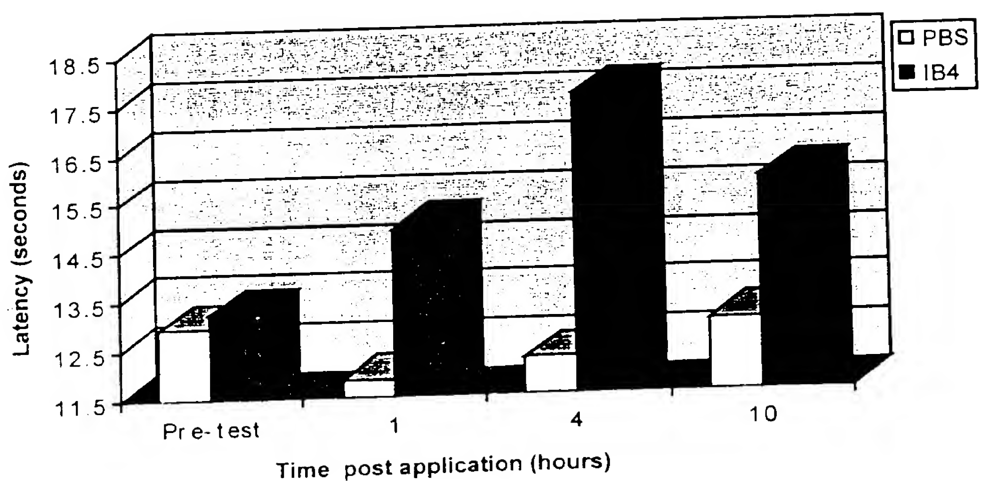

Figure 5.
Bandeirea simplicifolia lectin IB_4 is analgesic in a mouse hot plate model.

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